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Expression of size-selected mRNA encoding the intestinal Na/glucose cotransporter in *Xenopus laevis* oocytes

(*in vivo* translation/Na/glucose symporter/preparative electrophoresis)

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ABSTRACT **The expression of the rabbit intestinal brush-border Na/glucose cotransporter has been studied in *Xenopus* oocytes. Poly(A)$^+$ RNA isolated from the intestinal mucosa was injected into oocytes, and the expression of the transporter in the oocyte plasma membrane was assayed by measuring the Na-dependent phlorizin-sensitive uptake of methyl α-D-[^{14}C]glucopyranoside (MeGlc). Expression of the glucose carrier was detected 3–7 days after mRNA injection, and the rate of glucose transport was proportional to the amount of mRNA injected. mRNA (50 ng) increased the maximum velocity (V_{max}) of MeGlc uptake by as much as 10-fold over background. The total mRNA was fractionated by preparative agarose gel electrophoresis and each fraction was assayed for its ability to induce transport activity. The mRNA encoding the Na/glucose cotransporter was found in a single fraction of ≈2.3 kilobases (kb), which contained 3% of the total mRNA. A similar mRNA fraction (2.0–2.6 kb) isolated from colon did not induce expression of this transporter. *In vitro* translation of the fractionated intestinal mRNA showed enhanced synthesis of two protein bands at 57 and 63 kDa. The mRNA encoding the cotransporter is smaller (2.3 kb) than that (2.6–2.9 kb) encoding the 55-kDa facilitated glucose carrier in human hepatoma cells and rat brain.**

Active glucose absorption across the small intestine is performed by enterocytes at the tip of the villus. Transport occurs in two stages: the first is intracellular accumulation of the sugar across the brush-border membrane by a sodium/glucose cotransporter, and the second is facilitated diffusion of sugar out of the cell across the basolateral membrane. Recently, the sodium/glucose cotransporter has been identified as a 75-kDa polypeptide (1, 2), and some progress has been made in the characterization of this transport protein (3). The facilitated glucose carrier in the basolateral membrane is probably similar, if not identical, to the 55-kDa glucose carrier in human erythrocytes (see refs. 4–6).

Intestinal glucose absorption exhibits adaptive regulation with diet, starvation, diabetes, gestation, lactation, and aging (7). For example, in diabetes mellitus, the maximal velocity (V_{max}) of sodium-dependent glucose transport increases, and this is probably due to an increase in the number of sodium/glucose cotransporters. There is also a genetic component, and this is best exemplified by the rare hereditary disorder called primary glucose/galactose malabsorption (see ref. 8), which again is probably due to a defect in the cotransporter.

As a first step in cloning the gene for the intestinal sodium/glucose cotransporter, we have attempted to express the carrier in *Xenopus laevis* oocytes and to identify the size of the mRNA encoding the 75-kDa protein. Amphibian oocytes translate foreign mRNAs very efficiently (9) and have been successfully exploited to express functional membrane transport proteins ranging from ion channels to ion exchangers (10–12). Here we demonstrate that *Xenopus* oocytes successfully translate intestinal mRNAs and insert functional Na/glucose cotransporters into their plasma membranes. Furthermore, we have been able to size-fractionate intestinal mRNA by using preparative agarose gel electrophoresis, and we show that an mRNA fraction of ≈2.3 kilobases (kb) encodes the Na/glucose cotransporter. We conclude that the *Xenopus* oocyte provides a good model for the study of the expression of the intestinal brush-border glucose carrier and that preparative electrophoresis is a useful tool for the size-selection of functional mRNA.

METHODS

Oocytes were hand-dissected from ovarian fragments of *X. laevis*, treated with collagenase to remove follicular cells, and incubated in Barth's solution at 18°C (13). After 16–24 hr, the healthy oocytes were injected with 50 nl of mRNA (0.2–1 mg·ml^{-1}) or water (13) and incubated in Barth's solution with gentamicin at 18°C for another 3–8 days. Glucose uptake into oocytes was measured by a radiotracer technique. In brief, oocytes were incubated for 30 min in a Na-free buffer (100 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes/Tris, pH 7.5) and then placed in a Na (100 mM NaCl) or Na-free (100 mM choline chloride) buffer containing ≈20 μCi of methyl α-D-[^{14}C]glucoside (specific activity, 50–200 mCi/mmol; 1 Ci = 37 GBq; Amersham). After incubation for 15–90 min at 22°C, the uptake was terminated by removing the incubation medium and washing the oocytes with four 4-ml aliquots of ice-cold choline chloride buffer. Each oocyte was dissolved in 1 ml of 10% $NaDodSO_4$ and the ^{14}C was assayed by liquid scintillation counting. Sugar uptake was expressed as pmol per oocyte per hr and is presented as the mean ± SEM for four to seven oocytes.

RNA was isolated from rabbit small intestinal mucosa and from descending colon mucosa using a chloroform/phenol extraction (10, 11). Poly(A)$^+$ RNA was then selected by oligo(dT)-cellulose chromatography (Collaborative Research, Waltham, MA). For injection into oocytes, mRNA was dissolved in water (0.2–1 μg/μl).

Poly(A)$^+$ RNA was fractionated according to size using preparative agarose gel electrophoresis (14, 15). mRNA (300–500 μg) (≈1 μg/μl) was denatured by heating (2 min at 70°C) and rapidly cooled on ice. Separation was performed on a nondenaturing 1.5% agarose gel at 2°C, and the gel running buffer was 10 mM sodium phosphate buffer (pH 6.5). Aliquots of the fractions were precipitated with ethanol and analyzed on a 1% agarose minigel containing 2.2 M formal-

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Abbreviations: MeGlc, methyl α-D-glucopyranoside.

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dehyde (16). Individual fractions of the size range of interest were purified using Nensorb 20 cartridges (New England Nuclear). mRNA was eluted from the cartridges with 20% propanol and then precipitated with ethanol. The pellet was dissolved in water and assayed by *in vivo* and *in vitro* translation.

In vitro translation was performed using rabbit reticulocyte lysates (Promega Biotec, Madison, WI) in the presence of [^{35}S]methionine and canine pancreatic microsomes (New England Nuclear) as described by the manufacturers. Each reaction mixture contained 2 μg of poly(A)$^+$ RNA or 0.1 μg of fractionated mRNA. Aliquots of the translated proteins were separated on NaDodSO$_4$/10% polyacrylamide gels (16).

RESULTS

Preliminary experiments with noninjected *Xenopus* oocytes revealed that 50 μM D-[^{14}C]glucose uptake was linear from 15–90 min, but the rate was highly variable with oocytes from different animals (1–40 pmol per oocyte·hr^{-1}), and in 10 of 12 animals, the uptake was Na independent. This suggests a variable expression of the facilitated glucose carrier and little expression of the cotransporter in these cells. To minimize this variation, we used the glucose analog methyl α-D-glucopyranoside (MeGlc), which is handled by the Na/glucose cotransporter, but not the facilitated exchanger (4, 17). In seven experiments between September and November, the rate of 50 μM MeGlc uptake was 1.4 ± 0.3 and 0.2 ± 0.02 pmol per oocyte·hr^{-1} in Na and Na-free uptake media. This Na-dependent MeGlc uptake was completely inhibited by 0.5 mM phlorizin, the specific competitive inhibitor of Na/glucose cotransport (see Fig. 1). In one experiment, we measured the uptake of both D-glucose and MeGlc, and the rates were 9.3 ± 3.0 (n = 7) and 1.1 ± 0.2 (n = 7) pmol per oocyte·hr^{-1}. We therefore chose to use MeGlc to measure Na-dependent sugar transport owing to the low baseline flux and low variability among oocytes and among animals.

Injection of 50 ng of total mRNA extracted from the intestinal mucosa into oocytes resulted in a marked enhancement of MeGlc uptake. Fig. 1 shows the results in both water and mRNA injected oocytes of one experiment in which 50 μM MeGlc uptakes were measured in sodium, sodium and phlorizin, and in sodium-free incubation media. After 3 days

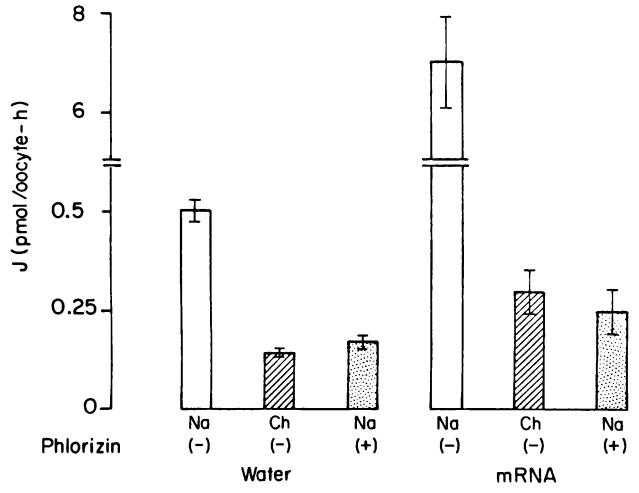

FIG. 1. Expression of rabbit intestinal MeGlc transport in *Xenopus* oocytes. Oocytes were injected with 50 nl of water or water containing 1 μg of rabbit intestinal poly(A)$^+$ RNA per μl. After 3 days of incubation, the uptake of 50 μM MeGlc was measured in the presence of 100 mM NaCl (open bars), 100 mM choline chloride (hatched bars), and 100 mM NaCl/0.5 mM phlorizin (stippled bars). Uptakes are presented as the means obtained with five to seven oocytes, and bars indicate SEM.

of incubation, the Na-dependent phlorizin-sensitive MeGlc uptake increased 22-fold from 0.3 to 6.7 pmol per oocyte·hr^{-1}. There was no change in the Na-independent (diffusional) MeGlc uptake. In seven experiments with different mRNA preparations over a 3-month period, injection of 50 ng total mRNA increased 50 μM Na/MeGlc cotransport 8(±3)-fold. Oocytes tested 5 days after mRNA injection showed another 1.6 ± 0.3 (n = 3)-fold increase in MeGlc uptake relative to that found after 3 days in mRNA-injected oocytes. The expression of Na-dependent MeGlc uptake at 3 days was proportional to the amount of mRNA injected. In one experiment, the slope was 0.1 ± 0.01 (SD) pmol per oocyte·hr^{-1}·ng^{-1}. mRNA isolated by the guanidine isothiocyanate method (16) also induced expression of the Na/glucose cotransporter in oocytes.

The kinetics of MeGlc transport were measured in both control and mRNA-injected oocytes. Uptake was measured as a function of MeGlc concentration (10 μM to 20 mM). The results showed that uptake occurred by both a saturable and a nonsaturable (diffusion) system. The nonsaturable process yielded a permeability coefficient of 2×10^{-9} cm·s^{-1} in control and injected oocytes. In one experiment, the maximal velocity of the saturable process (V_{max}) increased from 4.5 ± 0.9 (SD) to 30 ± 3 (SD) pmol per oocyte·hr^{-1} and the K_m decreased from 390 ± 100 (SD) to 100 ± 10 (SD) μM—i.e., injection of mRNA probably increased the number of Na/sugar cotransporters expressed in the plasma membrane by roughly an order of magnitude. Assuming that the turnover time of the cotransporter in oocyte plasma membranes is similar to that in intestinal brush borders (5 S^{-1}), the increase in V_{max} due to injection of mRNA is equivalent to the synthesis of 120 pg of transport protein (75 kDa) in 3 days (30 pmol per oocyte·hr^{-1}/18,000 hr^{-1} × 75,000). This amounts to <0.02% of the total protein synthesis per day (9).

mRNA was fractionated by electrophoresis (Fig. 2), and fractions were injected into oocytes. In the first screen of the fractions, the region between 2 and 4 kb doubled the rate of MeGlc transport compared to total mRNA. Next, the mRNA between 2 and 4 kb was divided into five fractions, and only mRNA with a size of 2.2–2.6 kb promoted MeGlc transport above background. Finally, eight individual mRNA fractions (fractions 26–33; Fig. 2) were injected (10–16 ng), and the results (Fig. 3) show that fraction 29 contained the mRNA encoding the cotransporter. This fraction contained 3% of the total mRNA eluted from the electrophoresis column (Fig. 2), and on formaldehyde/agarose minigels (Fig. 4), the mRNA in this fraction had an average size of 2.3 kb (range, 2.1–2.5 kb). Control experiments with mRNA from descending colon revealed that the mRNA encoding the cotransporter was tissue specific. Size-fractionated colon mRNA (2.0–2.6 kb) did not increase MeGlc uptake in oocytes above background (Table 1). However, the colon mRNA was able to produce other proteins as judged by the *in vitro* translation assay (Fig. 5).

Proteins produced by *in vitro* translation of both intestinal and colonic mRNA are shown in Fig. 5. Total intestinal mRNA yielded protein products ranging from 30 to 70 kDa, whereas the most abundant proteins synthesized from the mRNA of fraction 29 were in the range of 50–65 kDa. Detailed comparison of the translation products obtained from fractions 26–33 and from colonic mRNA (2.0–2.6 kb) suggested the enrichment of two protein bands at 57 and 63 kDa from the mRNA in fraction 29.

DISCUSSION

We have demonstrated that injection of intestinal mRNA into *Xenopus* oocytes results in the expression of functional Na/glucose cotransporters in the oocyte plasma membrane. In control oocytes, a small Na-dependent phlorizin-sensitive

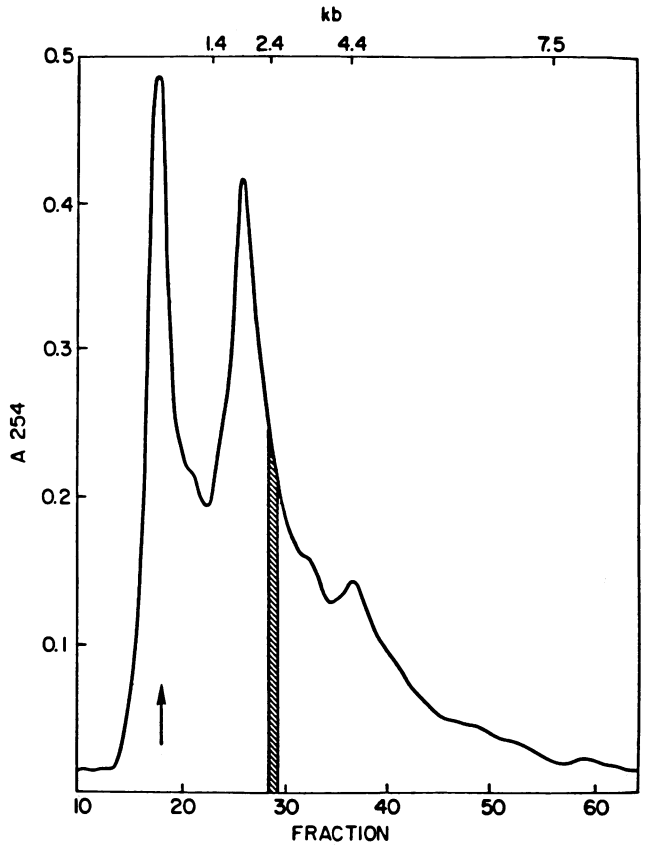

FIG. 2. Preparative agarose gel electrophoresis of intestinal mRNA. Heat-denatured poly(A)$^+$ RNA was electrophoresed at 300 V for 5 hr, and the optical density of the eluant was recorded at 254 nm. Samples were collected in 0.6-ml fractions. Tracking dye (bromophenol blue) appeared in fraction 18 (arrow) and mRNA was in fractions 20–60. Sample 29 (≈2.3 kb) contained the major fraction of the mRNA encoding the Na/glucose cotransporter. This fraction (hatched area) accounted for 3% of the recovered mRNA. The total recovery of mRNA in this experiment was 34%.

MeGlc uptake was detected, but the V_{max} for this system was 1/10th that found in oocytes injected with 50 ng of intestinal mRNA (Fig. 1). Parenthetically, the oocyte appears to be an exception to the rule that Na-dependent glucose transport is restricted to certain epithelia. The K_m of the intestinal glucose transporter was also significantly lower than the K_m for the native oocyte transporter (100 vs. 390 μM). The V_{max} for the intestinal transporter after 3 days of expression is ≈30 pmol per oocyte·hr^{-1} (≈100 pmol·cm^{-2}·hr^{-1} given oocytes 1 mm in diameter), but this is 1/100th to 1/1000th that calculated for the V_{max} for intact enterocytes (40 nmol per mg of protein, min^{-1} ≈60 nmol·cm^{-2}·hr^{-1}, given 2.5 × 10^{-7} mg of protein per enterocyte) (18).

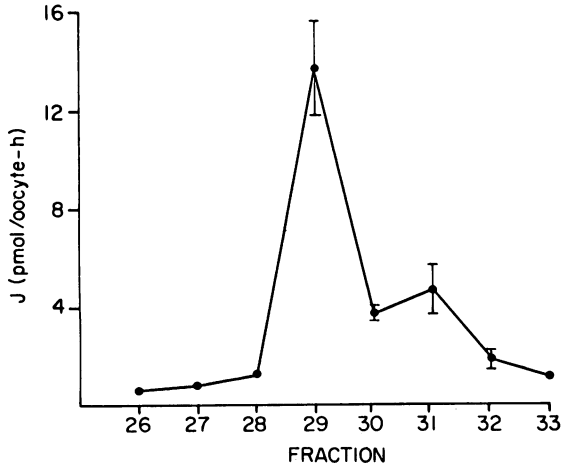

FIG. 3. Expression of MeGlc transport from size-fractionated intestinal mRNA. Purified mRNA from fractions 26–33 (Fig. 2) was injected into *Xenopus* oocytes (10–16 ng), and uptake of 50 μM MeGlc was measured 3 days later. Fractions 26–29 were injected into oocytes from one toad, and fractions 28 and 30–33 were injected into oocytes from another toad. The MeGlc uptakes were normalized according to fraction 28. Uptakes are shown as the means obtained with five to seven oocytes, and the SEMs are shown with the bars when they are larger than the size of the symbols.

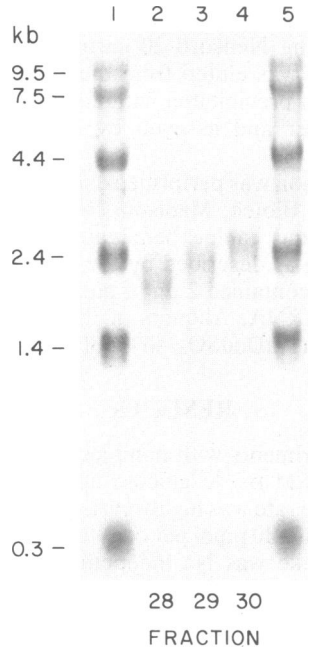

FIG. 4. Minigel of fractions 28–30 obtained from preparative agarose gel electrophoresis of intestinal poly(A)$^+$ mRNA (Fig. 2). A 1% agarose gel containing formaldehyde was run (16) and stained with ethidium bromide. Lanes: 1 and 5, standard RNA ladder (Bethesda Research Laboratories); 2–4, mRNA from fractions 28–30 obtained in the experiment shown in Fig. 2 and assayed for MeGlc transport in Fig. 3.

We also found that injection of intestinal poly(A)$^+$ RNA into oocytes resulted in the expression of the brush-border hydrolase trehalase. In a preliminary experiment, the activity increased from 2 ± 0.5 (n = 4) nmol per oocyte·hr^{-1} in H_2O-injected cells to 13 ± 0.5 (n = 4) nmol per oocyte·hr^{-1} in mRNA-injected [50 ng of poly(A)$^+$ RNA] cells after 4 days incubation. This indicates that the oocyte system is able to translate the mRNA encoding other brush-border membrane proteins.

Fractionation of intestinal mRNA using preparative agarose gel electrophoresis revealed that the mRNA encoding the Na/glucose cotransporter was enriched in a fraction of ≈2.3 kb. We were unable to detect any comparable activity

Table 1. Tissue specificity of Na/glucose carrier mRNA

	MeGlc uptake, pmol per oocyte·hr^{-1}	
	Na	Choline
Control	0.74 ± 0.13 (5)	0.12 ± 0.05 (5)
Colon mRNA	0.67 ± 0.06 (5)	0.02 ± 0.03 (3)
Intestinal mRNA	5.4 ± 1.3 (5)	0.16 ± 0.06 (5)

MeGlc uptake was measured 3 days after injection with 50 nl of water or 50 nl of water containing 50 ng of total intestinal mRNA. The colonic mRNA was size-selected (2.0–2.6 kb) by electrophoresis (see Fig. 2). Results are given as the mean uptake ± SEM with the number of estimates in parentheses.

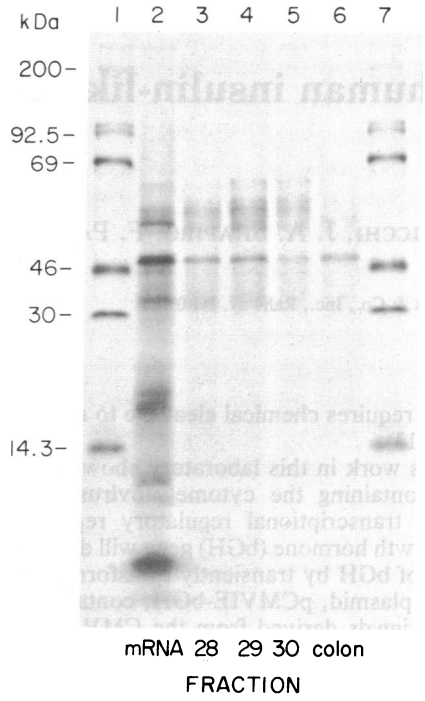

FIG. 5. *In vitro* translation of intestinal and colon mRNA using rabbit reticulocyte lysates in the presence of canine microsomes. $NaDodSO_4$/polyacrylamide gel electrophoresis of the ^{35}S-labeled protein products are shown (lanes 2–6) along with labeled standards (Amersham) in lanes 1 and 7. The molecular masses of the standards are indicated. Lane 2, the product of total poly(A)$^+$ mRNA from rabbit intestine; lanes 3–5, products from mRNA fractions 28–30 (Figs. 2 and 3); lane 6, products from 2.0- to 2.6-kb colon mRNA obtained by preparative electrophoresis (cf. Fig. 2). A control lane (not shown), run in the absence of mRNA, showed no synthesis of protein >30 kDa.

in size-fractionated mRNA from colon, and this is consistent with the lack of Na-dependent glucose transport in the colon. As shown in Figs. 2–5, electrophoresis provides a useful technique for fractionation of mRNA, which can then be assayed by *in vivo* and *in vitro* translation systems. The resolution of the system has enabled us to obtain an mRNA fraction of ≈2.3 kb containing 3% of the total mRNA, which contains the message encoding the proteins of interest. Adjacent fractions of ≈2.1 and ≈2.5 kb contained little of this mRNA.

The size of the mRNA encoding the 75-kDa Na/glucose cotransporter is smaller than the 2.6- to 2.9-kb mRNA, which encodes the 55-kDa facilitated glucose carrier in rat brain and human HepG2 hepatoma cells (5, 6). In these tissues, the glucose carrier mRNA contains a 1.5-kb region that encodes a 492-amino acid 55-kDa protein. The *in vitro* translation product is only 38 kDa, an unexplained discrepancy. There appears to be no homology between the facilitated and Na-dependent glucose carriers as (*i*) blot hybridization analysis of the fractionated glucose-transporter mRNA only reveals the presence of 2.9-kb mRNA in kidney (5, 6), (*ii*) genomic blot analysis with rat brain glucose transporter cDNA showed hybridization to a single gene at low stringency (6), and (*iii*) antibodies to the human erythrocyte-facilitated glucose carrier do not react with the renal Na/glucose cotransporter (19). The inverse relationship between the sizes of the two glucose transport proteins and their mRNAs could reflect differences in the size of the untranslated regions of the mRNAs and/or utilization of different polyadenylylation sites. Nevertheless, a 2.3-kb mRNA is not inconsistent with a Na/glucose cotransporter of 75 kDa.

This study clearly documents that the *Xenopus* oocyte may be used to study the translation of mRNAs encoding cotransport proteins and that electrophoresis may be used to fractionate functional mRNAs. The successful expression of intestinal mRNA for the glucose carrier also suggests a strategy for the isolation of the gene for this transport protein.

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